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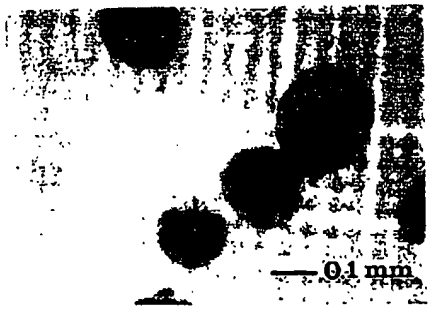
(54) Title: EMBOLIC MATERIALS COMPRISING OF CHITIN AND/OR CHITOSAN AND METHOD FOR PREPARING THEREOF



(57) Abstract: The invention relates to embolic materials used for embolization of blood vessels by inserting a catheter into the blood vessel and injecting the embolic materials through the catheter, and a method for preparing thereof. The embolic materials of this invention are comprised of chitin and/or chitosan particles having an average diameter of 50 to 1000 um and substantially spherical chitosan beads having an average diamter of 50 to 1000 um. The method for preparing chitosan beads comprises the step of making chitosan solution into microdroplet by pressurizing without passing in a capillary under a flowing inert gas stream and making beads by precipitating the above-mentioned microdroplet into coagulation solution. The embolic materials of this invention are substantially spherical and highly biocompatibility blood vessel embolic materials, which enable to embody a perfect embolization with little of foreign body reaction, to absorb and solidify medicinal agents such as contract media for angiography or anticancer agent without passing through a capillary, to be degradable or nondegradable in a blood vessel, to be prepared of a relatively uniform size in specific ranges, which has no oncogenicity and which are more or less inflammatory.

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EMBOLIC MATERIALS COMPRISING OF CHITIN AND/OR CHITOSAN AND METHOD FOR PREPARING THEREOF

FIELD OF THE INVENTION

The present invention relates to embolic materials comprising of chitin and/or chitosan, and method for preparing thereof, and more particularly, to functional embolic materials comprising of chitin and/or chitosan, which are harmless to humans, activation of immunity, gradually degradable in a blood vessel and which may be solidified a contrast media or an anticancer drug, and method for preparing thereof.

BACKGROUND OF THE INVENTION

Embolization generally is, when obstruction or devascularization of blood vessels in organs or tissues are needed clinically, to occlude blood vessel by inserting a catheter into blood vessels and injecting embolic materials therefrom. The embolic materials should effectively be occluded in the blood vessels at the treatment site, injected easily through the catheter and not pass through capillary vessels.

Recently, Polyvinylalcohol particles (refer to "PVA particles" hereinafter) (trade name : Contour, Target Therapeutics, U.S.A) has been used as particle embolic materials, and these occupies more than 80% of the world market. Although

PVA particle is a permanent embolic material, which is nonabsorbable to the human body, however, it has demerits such as blood vessel revascularization, difficulty in being obtained of a uniform size due to its irregularity of particle size and shape, clogging in the catheter, and its high costs.

Such blood vessel revascularization may cause a relapse of lesions, and such irregular in shape of embolic materials like PVA particles causes difficulty in embolization of the blood vessels at the treatment site and such embolic materials pass though the blood vessels of lesions and may cause a patient to die by embolizing of a distant organ's blood vessels (mainly, pulmonary artery).

As an actual clinical example, it has been reported that a patient died after embolization with PVA particles as an embolic material. Accordingly, it is necessary to develop an inexpensive spherical, oval or particle embolic material having a uniform size and shape and a perfect embolic effect.

The shells of crustaceans, such as crabs or lobsters discharged from food processing, contain a large quantity of natural polysaccharide. It is known that such polysaccharide is a natural polymer which is produced at the rate of one hundred billion ton in one year and is degraded by life on this planet. Chitin or chitosan, derived from the shells of crustaceans having high biocompatibility and little of antigen, is easily decomposed by an internal enzyme in the body, easily obtained as a natural material causing inexpensive production cost and has no tumor induction or toxicity, which are necessary conditions for embolic materials; thus attention has been paid to its use as a medical material recently.

Chitin is mucopolysaccharide as nitrogen-inclusive polysaccharide, which exists in the shells of crustaceans and as a constituent material of a cell membrane of

fungi. It exists as a form of glycoprotein in the human body and is obtained from removal of protein by an alkali treatment, and is not dissolved in water, organic solvent, or alkali solution. If it is hydrolyzed with acid, monosaccharide or oligosaccharide is obtained. Chitin is poly-N-acetyl-D-glucosamine which is polycondensed by β -(1→4) combination.

Meanwhile, chitosan is a β -poly-D-glucosamine which is obtained by separating acetyl from β -poly-N-acetyl-D-glucosamine by heating with a thick alkali solution, or melting alkali, and is a colorless noncrystalline powder and is not dissolved in water.

Korea is a country rich in high-quality chitin or chitosan resources. Recycling of waste into resources is of help to the protection of the environment, and furthermore, has an important meaning in a side view of the development of a new use of a new high-functional and high value added material.

According to the various recent research results, it has been reported that chitosan has medical benefits in antibacterial, inhibition of tumor metastasis, anti-AIDS activity, anti-blood coagulation activity, reduction of blood cholesterol, and the cure of a burn, and the like. Even chitinous substance in a cuttlebone is so highly biocompatible that its powder has been used as a hemostatic, or a treatment, for injury in the past. At present, it is being used clinically for an antipyrotic or bioabsorbable suture in Japan and Russia, and it is expected that the scope of its application will be expanded in the future.

Examples of the typical embolic materials which are currently used widely are gelfoam, PVA particle, coil, split balloon, anhydrous ethanol, N-butyl 2-cyano

acrylate (NBCA), and the like. When it comes to choice of an embolic material, it is necessary to consider various mediation variables such as the period of occlusion of the blood vessel at the tretment site, the shape of embolic materials, namely liquid or solid particles, the type of accompanying complications, the facility of embolization procedure and various parameters, and the like, depending on the kinds of diseases.

However, any embolic material which satisfies all of the ideal conditions has not yet been reported. Currently, it is reported that PVA particles are most widely applied clinically. For the clinical example, uniform particle sizes, which are obtained by grinding PVA larger particles, are used.

Typical examples of commercial PVA particles which are most widely being used are Contour®(PVA, Target Therapeutics, USA) and Ivalon® (Laboratories Ingenor Inc., Paris), which occupies more than 80% of the world market. These kind of embolic materials are the permanent particle embolic materials, which are nonabsorbable in the human body, and due to the irregularity of particle size and shape, it is difficult to obtain their distribution of a relatively exact average particle size compared to a spherical body, which makes it more difficult to accomplish an embolization to the blood vessel at the treatment site.

As to a problem, which the irregularity of particle size and shape may cause, a clinical example has been reported that the embolic materials had moved to a distant organ (mainly pulmonary artery) and aroused any embolization, which resulted in a patient's death. The solid particle embolic material, which is biodegradable, are auto-thrombus, gelform, and the like, whereas the permanent embolic materials, which are non-biodegradable, are PVA particle, silicon sphere,

glass, and the like. However, the solid particle embolic materials has several problems such as clogging in the catheter, obstruction of the catheter, irregularity of particle size, and the like.

SUMMARY OF THE INVENTION

Accordingly, the first object of the invention is to prepare oval or spherical embolic materials having relatively uniform particle size, which have a perfect and efficient embolic effect in any blood vessels.

The second object of the invention is to prepare embolic materials with high biocompatibility without any adverse reactions because it is not possible to induce tumor.

The third object of the invention is to prepare embolic materials which can be suspended as respective particles without agglutination in a salt solution in order to be easily injected through a catheter.

The fourth object of the invention is to prepare embolic materials which may be easily delivered through a catheter in a solution as a carrier.

The fifth object of the invention is to prepare embolic materials which may efficiently occlude the blood vessel to be embolized.

The sixth object of the invention is to provide embolic materials which do not pass through capillary vessels.

The seventh object of the invention is to provide blood vessel embolic materials which can cause more or less inflammation in any vessels and as a result, easily perform embolization.

The eighth object of the invention is to provide blood vessel embolic materials which are degradable and absorbable with the embolic materials, or not, with the lapse of time after embolization.

The ninth object of the invention is to provide functional embolic materials which can immobilize contrast media and/or anticancer agent.

The tenth object of the invention is to provide a method for preparing the embolic material according to the foregoing first to ninth objects.

According to one aspect of the invention in order to achieve the foregoing first to ninth objects, it is to provide substantially spherical embolic materials comprising of chitosan.

According to a further aspect of the invention in order to achieve the foregoing second to ninth objects, it is to provide embolic materials comprising of chitin and/or chitosan particles.

According to an additional aspect of the invention, it is to provide substantially spherical functional embolic materials comprising of chitosan and/or chitin, which are solidified with contrast media and/or anticancer agent.

In accordance with a further aspect of the invention in order to achieve the foregoing tenth object, it is to provide a method for preparing chitosan beads comprising the step of: passing chitosan solution of lactic acid, acetic acid, or hydrochloric acid through a capillary blowing air under pressure to obtain chitosan microdroplet of a desired size, and then precipitating it into a solidifing solution to form chitosan beads, which solidify the chitosan into the uniform particle size and shape.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A and Fig. 1B are, respectively, a whole and an essential part schematic view for explaining the method for preparing embolic materials according to the invention.

Fig. 2 is an enlarged microphotograph of the conventional PVA particle having an average diameter of 150 to 250㎛.

Fig. 3A and Fig. 3B are, respectively, an enlarged microphotograph of a chitin and a chitosan particle having an average diameter of 150 to 250㎛ according to the invention.

Fig. 4A and Fig. 4B are, respectively, a microphotograph and a naked eye picture of a chitosan bead having an average diameter of 150 to 250㎛ according to the invention.

Fig. 5 is an enlarged picture of functional chitosan embolic material according to the invention with catheter and solidification of Doxorubicin · HCl as an antineoplastic agent.

Fig. 6A and Fig. 6E are pathological report pictures of rabbit renal showing the results of experimental example 7, respectively.

DETAILED DESCRIPTION OF THE INVENTION

Blood vessel embolic materials comprising of chitosan and/or chitin of the present invention are polymers derived from nature, which are harmless to human body, activation of immunity, and are gradually degradable in a blood vessel.

Referring to the chitin and chitosan particles used in the present invention, although it is not intended to be limited regarding chitin's number average molecular weight, it is the range of about 400 thousand to 1 million and chitosan number average molecular weight is the range of about 20 thousand to 400 thousand, preferably about 100 thousand to 200 thousand although it is not intended to be limited regarding it. Also, it is desirable to use chitosan whose deacetylation degree is more than 60% although it is not intended to be limited regarding it.

When preparing chitosan beads of the invention, there is some apprehension that the beads are not formed smoothly if the number average molecular weight is below 20 thousand. The viscosity of chitosan when forming chitosan beads is the range of 2 to 100cps, preferable of 5 to 86cps, the most preferable of 5 to 20cps, and the concentration of chitosan is the range of 0.5 to 10.0%(W/V), preferable of 1.5 to 5.5%(W/V), the most preferable of 4.0 to 5.5%(W/V). When it excesses the above-mentioned range, there is a possibility to prepare chitosan beads with inferior suspension properties and non-spherical shapes.

The blood vessel embolic materials comprising of chitosan and/or chitin of the present invention are, in concrete terms, chitosan and/or chitin particles of a relatively uniform average diameter in specific ranges, preferably spherical chitosan beads of a relatively uniform average diameter in specific ranges, which are able to suspend for a constant time in a salt solution only or mixture with a contrast media

or an anticancer agent, which the suspended particles do not clump up to each other, which pass through a catheter easily, which easily occlude the blood vessel to be embolized, and which do not pass through a capillary.

The blood vessel embolic materials comprising of chitosan and/or chitin of the present invention may be formed as chitosan and/or chitin particles having a uniform particle size range by sieving after grinding chitosan and/or chitin, preferably as spherical beads having a relatively uniform average diameter in specific ranges by dissolving chitosan in an appropriate solvent.

The detailed description of blood vessel embolic materials of the invention and method for preparing thereof are as below:

Although there is no intention to be limited on the average diameter of particles comprising of chitin and/or chitosan used in the invention, it is the range of 50 to 1000㎛, or 150 to 500㎛, or 150 to 250㎛, or 250 to 355㎛, or 355 to 500㎛. Such chitin and/or chitosan particles are able to be isolated in order to have an average particle size of a specific range by grinding and subsequent sieving, which can easily be performed by those skilled in the art; therefore, the specific explanation is omitted hereon.

In addition, the viscosity and the concentration of chitosan in preparing chitosan beads preferably used for the invention, are important factors to form spherical embolic materials, and the range thereof are as mentioned above. It is desirable to wash the embolic materials of the form of beads according to the invention, with distilled water several times in order to remove solidification agents such as a sodium hydroxide solution.

Furthermore, the storage stability and the utility convenience of the

embolic material, according to the invention, may be improved by freeze-drying in a temperature range of - 20 to - 80℃, or in liquid nitrogen.

Fig. 1A and Fig. 1B are, respectively, a whole and an essential part of a schematic view for explaining the method for preparing embolic materials according to the invention and an apparatus for preparing the blood vessel embolic materials of the invention includes a solution storage bath (not shown in the drawings), a carrying conduit 3 of chitosan solution, an air carrying conduit 6, several micro needle-shaped capillaries 5 branched from the conduit 3, and a solidification bath 8.

The chitosan bead 1 for the embolization according to the invention is prepared by dissolving chitosan having specific ranges of the deacetylation rate and the viscosity in lactic acid, acetic acid or hydrochloric acid solution with a concentration of 1 to 10%(w/v) and filtering it, and then compulsorily transferring the solution 10 by a pump 2 through the conduit 3 and the micro needle-shaped capillary 5 having the inside diameter of 50 to 1000㎛, which is severally branched from the conduit 3.

At this time, it is possible to form a microdroplet from the solution, which is pushed out from the above capillary by compulsorily blowing air 11 (or inert gas such as nitrogen or argon) through the air-carrying conduit 6 and, at the same time, to control the size of beads.

When the injecting amount of air is increased, the size of the beads becomes relatively smaller, and on the contrary, when the injecting amount of air is reduced, the size of the beads becomes relatively larger. In Fig 1, the numeral number 4 denotes a capillary supporter.

The chitosan beads 1 for embolization according to the invention pass

through the capillary 5 and then are loaded in the solidification bath 8. As to the solidification solution in bath 8, it is necessary to select one or a mixed solvent, to show the high solidification speed of chitosan and to prepare more perfect spherical chitosan beads. Such preferable examples are a mixture of water, ethanol and sodium hydroxide or potassium hydroxide, although the invention is not limited thereby, and it is possible to use these appropriate solvents individually, or mixing optionally.

In case of using a mixed solvent of sodium hydroxide, ethanol and water, the mixture ratio may be a range of 1 : 0 ~ 18 : 4 ~ 24. The chitosan solution 10 is coagulated in the solidification bath 8 and formed as beads for embolization of the invention. Although the intention is not to be limited on the average diameter of beads of the invention, it may be a range of 0 to 1000㎛, or 150 to 500㎛, or 150 to 250㎛, or 250 to 355㎛, or 355 to 500㎛.

The chitosan beads of the invention are required to have a superior suspension in a salt solution, a mixed solution of a salt solution and a contrast media and/or an anticancer agent, which guarantees a good passing property in the catheter. It is desirable that the chitosan bead embolic materials of the invention are able to suspend in a physiological salt solution for longer than 30 minutes if the diameter of a bead is 50 to 350㎛, and for longer than 5 minutes if it is 350 to 500㎛ or more.

Furthermore, the preferable viscosity of chitosan liquid when preparing chitosan beads of the invention is a range of 5 to 86cps, and the preferable concentration of chitosan is generally a range of 1.5 to 5.5%(W/V), although it depends on the density respectively . In addition, it is desirable that the embolic

materials have weak cohesion in a physiological salt solution or a mixed solution with other substances, and the embolic materials of the invention do not clump up in a physiological salt solution and a mixed solution with the contrast media.

In the interim, the chitin and/or chitosan particle of the invention is principally irregularity, but smoothly passes through a catheter in a carrying solution as a carrier and tend to clog the catheter, because, it is assumed, the penetration of water is noteasily into the inside of particles and, since the amino group is not positioned outside, the static electricity load is small.

Furthermore, if necessary, it is possible to cross-link using a cross-linking agent such as hexamethylenediisocyanate (HMDC), glutaraldehyde, epichlorohydrine or mixtures thereof in order to be non-hydrolysis or difficult to be hydrolysis in the blood vessel. Of this cross-linking agent, the most preferable one is hexamethylenediisocyanate (HMDC). In case of using HDMC as a cross-linking agent, the cross-linking agent can be used within a range of 10% of chitin and/or chitosan weight.

To be more detailed, for instance, it is possible to substitute ethanol for a certain amount of chitosan and/or chitin embolic materials, substitute dimethylformamide (DMF) for several times, add HDMC in the presence of DMF and precipitate them for 2 to 48 hours, stirring intermittently, wash sufficiently the chitosan and/or chitin embolic materials by a mixed solvent of ethanol and water, and then sterilize. If necessary, it is possible to select them according to size after freeze-drying and sieving.

In case of using glutaraldehyde as the cross-linking agent, the cross-linking process can be carried out by adding chitosan beads to acetic acid liquid, adding

glutaraldehyde to the above solution, stirring it for 24 hours under room temperature, and then reducing the schiffe base by adding $NaBH_4$ until pH is adjusted to be neutral.

In case of using epichlorohydrine as the cross-linking agent, it is possible to cross-link by adding chitosan beads to sodium hydroxide solution and then adding epichlorohydrine to it and then stirring it for 24 hours under room temperature.

In addition, the chitosan and/or chitin embolic materials of the invention could be prepared to functional embolic materials, which absorbes various kinds of contrast media for angiography or anticancer agent. For example, since the contrast media is absorbed and solidified to the embolic materials of the invention by partially cationizing the chitosan and/or chitin, substituting ethanol for it, adding a certain amount of contrast media, or anticancer agent to it and then removing ethanol from it under vacuum or reduced pressure, it is possible to obtain a radiograph of embolic materials by irradiation with radiation or to absorb and solidify the anticancer agent.

While the invention has been described in detail with reference to a preferred embodiment thereof, it will be apparent to one skilled in the art that various changes and modifications can be made, and equivalents employed, without departing from the spirit and scope of the invention.

The present invention will be further clarified by the following examples and comparative examples, which are intended to be exemplary of the present invention. The invention is not intended to be limited to the examples shown.

Example 1

Chitin and chitosan, which were purchased in a flake state were boiled in a

sodium hydroxide solution to remove remaining protein, and to remove heavy metals, which may have remained, for 5 hours with a 2% sodium ethylenediaminetetraacetate(EDTA-4Na), washed by a neutral detergent, dried, intermittently ground by a grinder (Hoodmixer FM-681, Han-il Electricity Co., Ltd., Korea), and then sieved by a micro sieve (Chung-Kye Industries, Ltd., Korea) in order to obtain particles of a uniform size.

As a results, chitosan and chitin particles having an average diameter of 150 to 250㎛ were obtained. The microphotographs of the obtained chitosan and chitin particles are represented in Fig. 3A and 3B, respectively.

Comparative Example 2

Polyvinylalcohol(PVA) (tread mark: Contour, Target Therapeutics, USA)(average particles diameter: 150 to 250㎛), which is widely used as an embolic material from early time, was commercially purchased. The microphotographs of PVA are represented in Fig 2A.

As shown in Fig. 2A, it was confirmed that PVA has an irregular size and shape.

Examples 2 to 18

Chitosan beads or chitosan coagulant were prepared by the apparatus for manufacturing chitosan beads as illustrated in Fig. 1A and Fig. 1B, using various chitosan solutions whose chitosan number average molecular weight is more than 20,000, whose deacetylation degree is more than 60%, whose chitosan concentration is in the range of 1.5 to 5.5%(w/v), whose acetic acid concentration is 4%, whose chitosan viscosity is in the range of 5 ~ 86cps, and using a mixed solvent of sodium

hydroxide/ethanol/water=4/20/76 (w/v/v) as a solidification agent. These are Examples 2 to 18, respectively.

In regard to the chitosan coagulant obtained from the above Examples 2 to 18, the shape under wet conditions, the reduction shape after the drying step, solubility in 10% acetic acid solution, suspension, catheter passage, size and the like are measured. The results are shown in Table 1.

The mark X on the shape indicates a case of not forming beads while the mark O indicates a case of forming beads. Suspending properties were decided by suspended time in a mixed solution of a physiological salt solution (2.5ml) and contrast media (2.5ml, Trade name: Hexabrix, containing ioxaglate sodium, 320mg I/ml, Taejoon Pharm., Co., Ltd, Korea) at the ratio of 1 : 1, filled in a 5㎖ syringe; the mark O indicates a case of suspended time for longer than 30 minutes if the average diameter is 50 to 350㎛, and for longer than 5 minutes if it is more than 350㎛. Catheter passage is decided on whether or not to pass without causing obstruction due to cohesion inside a catheter.

Fig. 4A and Fig. 4B are a microphotograph and a naked eye photograph of chitosan beads of Example 13, respectively.

Examples 19 to 25 and Comparative Example 2

The same procedure as in Examples 2 to 18 was effected except that the chitosan solution having 9.8 cps of the chitosan viscosity, 4%(w/v) of the chitosan concentration and 4% of the concentration of acetic acid, and the components as indicated in Table 2 as the solidification agent was used, and then the chitosan beads and the chitosan coagulant were prepared.

The composition of the coagulant and the composition ratio, and the results are set forth in Table 2.

Experimental Example 1: embolization for rabbit renal

Twenty four healthy New Zealand White rabbits of 2.0 to 3.5kg in weight without separating males and females were classified into four groups, 6 rabbits to each group. 150 to 250㎛ of CONTOUR®(polyvinyl alcohol, Target Therapeutics, USA) of Comparative Example 1 as control group were used in group 1, 150 to 250㎛ of chitin particles of Example 2 in group 2, 250 to 355㎛ of chitosan particles of Example 2 in group 3, 150 to 250㎛ of chitosan beads of Example 7 in group 4, 250 to 355㎛ of chitosan beads of Example 8 in group 5, and 355 to 500㎛ chitosan beads of Example 16 in group 6, respectively. The chitosan bead was cross-linked according to the method as described in Example 27.

Rabbits were anesthetized by an intramuscular injection of 35 mg/kg of Ketara®(Ketamine, Yuhan Corporation, Seoul, Korea), and 5 mg/kg of Rompun®(Xylazine, Bayer Korea, Seoul, Korea). A 4F angiography cobra catheter (Terumo, Tokyo, Japan) was inserted through the right femoral artery and the right renal artery was selected. The contrast media was injected into the renal artery under the irradiation of radiation to examine angiography. Subsequently, the embolization of the renal artery was continued by the prepared embolic materials until complete occlusion of a blood vessel was accomplished. Throughout the three days after the experiment, 100mg/kg of Ceftexole Sodium®(Shin Poong Pharm. Co., Ltd, Seoul, Korea) was daily injected by an intramuscular injection.

A rabbit from each group, one by one, sacrificed on the first day, the third

day, the first week, the second week, the fourth week, and the eighth week. Both renal of the rabbit were taken out and solidified in a 10% of formalin solution and then HE dyed. Hematological examination (CBC) and biochemical examination were conducted on the rabbit's blood extracted before embolization and at the time of sacrifice. Hematological examination was analyzed on leucocyte, erythrocyte, blood platelet, hematocrit and hemoglobin, and found the percentage of leucocyte. Biochemical examination was conducted on BUN, creatinine, total bilirubin, GOT, GPT, alkaline phosphatase, total protein, albumine, A/G ratio, LDH, CPK, glucose, total cholesterol, amylase, electrolyte (Na, K, Cl).

As to the results, the obstruction of q catheter due to embolic materials in examination on a catheter during and after the operation in groups 1, 2 and 3 was not observed. In group 4, the cloggong of a catheter in examination on a catheter after the operation in one sample (16%) was observed. Embolization was successful on all the 24 rabbits. The pathological report with the naked eye indicated that in PVA (group 1) case, a hard and contracted renal was observed after the second week. With chitosan beads, it was observed on the fourth week in group 2, the eighth week in group 3, and the fourth week in group 4, respectively.

Embolic materials were not absorbed until the eighth week. It was observed that blood vessels were occluded by the embolic materials, and it showed an acute inflammation change and an organization, and then turned back to revascularization. The embolic materials showed a coagulation necrosis and were contracted making calcification and fibrosis. Compared with the case of PVA (group 1), the coagulation necrosis occurred later in the group of chitosan beads (groups 2, 3, 4), the beads became inflamed later and showed more severe vacuities than the

case of PVA. There was no significant difference depending on the size of beads. Since chitosan beads have not been observed in the embolized renal after the tweentieth week, it was found that chitosan is degradable in blood vessels.

In a blood biochemical examination, it was observed that BUN and creatinine for showing the function of the renal increased until the first week after the operation in all four groups. This coincides with a case that on another renal was embolized due to regurgitation during the operation. Total bilirubin, GOT, GPT, and alkaline phosphatase all showed normal findings. CPK and LDH have increased; but it was the same in the case of PVA. There was no increase of eosinophil, which may occur with an allergy.

Using chitosan beads prepared by the aforementioned method, it proved successful to intercept blood flow by inserting a catheter through the rabbit's right femoral artery and injecting embolic materials. The embolized renal of the rabbit resulted from the test is shown in Fig. 6A to Fig. 6E, and the pathological findings on the rabbit renal is set forth in Table 3 and Table 4, respectively.

Since chitin and/or chitosan particles and chitosan beads which are natural biopolymers of the present invention are activation immunity, when they are injected into a blood vessel for embolization; it was observed that the leucocyte count increased between the fourth week and the eighth week, whilst it decreased in the case of PVA reversely.

In addition, while revascularization occurred after around one week in the case of PVA, it did not occur, or at least it was insignificant, in the case of embolic materials of the present invention. Furthermore, since the embolic materials of the present invention, especially chitosan beads, cause vasculitis, thickening of vessel

wall occurs, which results in gradual stricture and occlusion of blood vessel lumen; therefore, it is possible to permanently occlude the desired blood vessel with the intention of embolic materials.

According to the present invention, since chitin and/or chitosan particles and chitosan beads are natural biopolymer, they are highly biocompatible, activation of immunity and biodegradable. Particularly, since chitosan beads are spherical in shape, they seldom pass through a capillary vessel and may easily be embolized a designated portion due to their uniform size. Moreover, they have excellent catheter passage (utility).

Table 1

	Concentration of chitosan in acetic acid (w/v)	Viscosity (cps)	Shape under wet condition	Reduction shape after drying step	solubility	Suspending properties	Catheter passage	size(㎛)
Example 2	1.5%	86	X	-	X	O	O	50-1000
Example 3	1.8%	86	O	X	X	O	O	50-1000
Example 4	2%	86	O	X	X	O	O	50-1000
Example 5	2.5%	86	O	X	X	O	O	50-1000
Example 6	3%	10	X	-	X	O	O	50-1000
Example 7	3.3%	9.4	O	O	X	O	Pass below 500㎛	Below 150/150-212/ 212-250
Example 8	3.5%	9.4	O	O	X	O	Pass below 500㎛	Below 150/150-212/ 212-250/250-355
Example 9	3.7%	9.4	O	O	X	O	Pass below 500㎛	Below 150/150-212/ 212-250
Example 10	4%	5.8	X	-	X	O	O	50-1000
Example 11	4%	9.8	O	X	X	O	O	50-1000

Table 1(continued)

	Concentration of chitosan in acetic acid (w/v)	Viscosity (cps)	Shape under wet condition	Reduction state after drying step	solubility	Suspending properties	Catheter passage	size(㎛)
Example 12	4%	6.4	X	-	X	O	O	50-1000
Example 13	4.5%	10.2	O	O	X	O	Pass below 500㎛	50-1000
Example 14	4.5%	9.8	O	O	X	O	Pass below 500㎛	50-1000
Example 15	4.5%	6.4	X	O	X	O	-	250-355/355-500
Example 16	5%	10.2	O	O	X	O	Pass below 500㎛	250-355/355-500
Example 17	5%	9.8	O	O	X	O	Pass below 500㎛	50-150/150-250/250-355/355-500/500-710
Example 18	5.5%	10.2	X	-	-	-	-	-

Table 2

Example	Example 19	Example 20	Example 21	Example 22	Example 23	Example 24	Example 25	Example 11	Canparative Example 2
Etanol	0	10	20	30	40	50	60	70	80
Water	96	86	76	66	56	46	36	26	16
Sodium hydroxide or Potassium hydroxide	4	4	4	4	4	4	4	4	4
Results	Good	Good	Good	Good	Good	Good	Good	Good	Inappropriate

Table 3 : Pathological finding of embolized renal

Term / Group	One day	3rd days	First week	2nd weeks	4th weeks	8th weeks	24th weeks	32nd weeks
Group 1 PVA (150-250㎛)	Swelling Some discoloration Soft Smooth	Swelling Some discoloration Soft Smooth	Normal Discoloration Soft Smooth	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node
Group 2 Chitin particles (150-250㎛)	Swelling Some discoloration Soft Smooth	Swelling Some discoloration Soft Smooth	Normal Discoloration Soft Smooth	Normal Discoloration Soft Smooth	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node
Group 3 Chitosan particles (150-250㎛)	Swelling Some discoloration Soft Smooth	Swelling Some discoloration Soft Smooth	Normal Discoloration Soft Smooth	Some constriction Discoloration Hard Soft-node	Constriction Discoloration Hard Soft-node	Constriction Discoloration Hard Node	Constriction Discoloration Soft Atrophic node	Constriction Discoloration Hard Node
Group 4 Chitosan beads (150-250㎛)	Normal Some discoloration Soft Smooth	Swelling Discoloration Soft Smooth	Swelling Discoloration Soft Smooth	Some constriction Discoloration Soft-hard Some node	Constriction Discoloration Soft-hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Soft Atrophic node

Table 3(Continued)

Term Group	One day	3rd days	One week	2nd weeks	4th weeks	8th weeks	24th weeks	32nd weeks
Group 5 Chitosan beads (250-355㎛)	Swelling Free discoloration Soft Smooth	Swelling Some discoloration Soft Smooth	Some small Some discoloration Soft Smooth	Some small Some discoloration Soft Smooth	Some small Some discoloration Soft Smooth	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node
Group 6 Cross-linked chitosan beads (355-500㎛)	Swelling Free discoloration Soft Smooth	Swelling Some discoloration Soft Smooth	Swelling Discoloration Soft Smooth	Some small Discoloration Soft Soft-node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node	Constriction Discoloration Hard Node

Table 4 : Microscopical and pathological finding of embolized renal

Term / Group	One day	3rd days	One week	2nd weeks	4th weeks	8th weeks	24th weeks	32nd weeks
PVA (150-250㎛)	PMN bleeding, Coagulation necrosis	PMN organic Bleeding, Vasculities, Coagulum organizing	Coagulum organizing, Revascularization of blood vessels, Chronic reaction, Fibrosis, Giant cell, Diffusible necrosis	Coagulum organizing, Revascularization of blood vessels, Fibrosis, Calcification, Fibrous tissue, Diffusible necrosis	Coagulum organizing, Revascularization of blood vessels, Fibrosis, Calcification, Fibrous tissue, Diffusible necrosis	Diffusible constriction, Fibrous tissue	Revascularization, Giant cell	All necrosis, Fibrous tissue
Chitin particles (150-250㎛)	Diffusible bleeding, Acute Reaction, Vasculities, coagulation necrosis	Vasculities Acute reaction, Coagulation necrosis	Cuneiform necrosis, Vasculities	Calcification(+)	Calcification(++)	Foreign body granuloma (+++)	FB(Giant cell) Xanthoma cell, Tunica subintimal Fibrous forming, Lumen occlusion	FB reaction Xanthoma cell
Chitosan particles (150-250㎛)	Congestive bleeding, Vasculities, coagulation necrosis is not discovered.	Acute cell reaction, Additional coagulation necrosis	Difinite cuneiform necrosis, Coagulum organizing Revascularization of blood vessel, Calcification Chronic reaction	Coagulum organizing Diffusible necrosis	Foreign body reaction, All necrosis	Foreign body reaction (++), Bead absorption	Giant cell, Tunica subintimal, Fibrous forming, Lumen constriction, Focal revascularization of blood vessel, Bead absorption	All necrosis, Fibrous tissue

Table 4(Continued)

Term / Group	One day	3rd days	One week	2nd weeks	4th weeks	8th weeks	24th weeks	32nd weeks
Chitosan beads (150-250㎛)	No cell reaction	Vasculities, Coagulation necrosis	Necrotic Vasculities, Cuneiform necrosis, Infarct	Foreign body reaction(+) Coagulum organizing (-)	Contractility calcification	Diffusible fibrosis	All necrosis and fibrosis, Chronic inflammatory cell, Infiltration, Giant cell, Bead absorption	All necrosis and fibrous organizing, Xanthoma cell, Bead absorption
Chitosan beads (250-355㎛)	No cell reaction, Lumen bleeding	Necrotic vasculities, No infarct	Necrotic vasculities, Cuneiform necrosis. Calcification	Vasculities, Diffusible necrosis	Diffusible necrosis, No fibrous organizing	Acute reaction, Bead absorption	Tunica subintimal, Fibrous forming, Lumen constriction, Bead absorption	No FB reaction, Tunica subintimal, Fibrous forming Lumen constriction, Bead absorption
Cross-linked chitosan beads (355-500㎛)	No cell reaction	Vasculities Local bleeding	Necrotic vasculities, Calcification, Coagulation necrosis	Vasculities Calcification Coagulation necrosis	Diffusible necrosis, No fibrosis	Diffusible necrosis	FB reaction, Revascularization of blood vessel, Bead absorption	No FB reaction Bead absorption

Example 26 : Method for preparing freeze-dried chitosan embolic materials

Example 17 shows the method for preparing wet chitosan embolic materials, and they were freeze-dried at -40℃, sieved, and then sized for convenient storage.

Example 27 : the preparation of cross-linked chitosan embolic materials

Beads prepared in Example 26 have been washed by water to neutralize its pH and ethanol was substituted, and then 50ml of that has been dried, wherein its dry weight was 1.6g. It is necessary to cross-link this bead since it has weak intensity and is not easily restored to a round-shape after freeze-drying and it is dissolved in an acid solution. To be cross-linked, beads which had been substituted with ethanol have been substituted DMF and added to 0.1 mol of HMDC per 1 mol of the amino group of chitosan. Then, it has been stirred slowly for 12 hours at room temperature. Any remaining have been removed by thorough washing using DMF and water. This bead was undegradable in a 10% acetic acid solution, and the introduction of a cross-linking agent was confirmed through 13C-NMR spectra. The cross-linking degree was about 10% and it was calculated by weight change before and after cross-link against a certain amount of chitosan bead. It is assumed from this result that the whole amount of the HMDC added has been used for cross-link.

Example 28 : the preparation of chitosan embolic materials solidified to ioxaglic acid (trade name; Hexabrix, main ingredients; ioxaglic acid, contrast media for angiography, 320mg I/ml, Taejoon Pharm., Co., Ltd, Korea)

Since a radiograph cannot be obtained by chitosan itself, sodium ioxaglate contrast media for angiography has been added and then has been absorbed, and as a result, a radiograph of chitosan embolic materials has been obtained.

Embolic materials, of which sodium ioxaglate has been absorbed and solidified, have been obtained by adding 8ml of ethanol to 100mg of chitosan embolic material of Example 26, substituting it three times under vacuum, and adding 10ml of Hexabrix® thereto, and then removing ethanol and moisture under vacuum. This contrast media for angiography is absorbed amino groups of chitosan and carboxyl groups of contrast media by ion exchange. It also applies to chitosan beads of Examples 17 and 27.

Example 29 : the preparation of chitosan embolic materials solidified to ioxitalamic acid contrast media for angiography (trade name; Telebrix, main ingredients; ioxitalamic acid, 300mg I/ml, Taejoon Pharm., Co., Ltd, Korea)

Embolic materials, of which ioxitalmic acid has been absorbed and solidified, have been obtained by adding 5ml of ethanol to 100mg of chitosan embolic material of Examples 17, 26, and 27, substituting it twice under vacuum, adding 6ml of Telebrix thereto, and then removing ethanol and moisture under vacuum. This contrast media for angiography is absorbed amino groups of chitosan and carboxyl groups of contrast media by ion exchange. It also applies to chitosan beads of Examples 17 and 27.

Example 30 : the preparation of chitosan embolic materials solidified to Iohexol (trade name: Omnipaque, main ingredients: Iohexol, 350mg I/ml, Nicomed Imaging A.S., Korea) contrast media for angiography

Embolic materials, of which omnipaque has been absorbed and solidified, have been obtained by adding 3ml of ethanol to 100mg of chitosan embolic material of Examples 17, 26, and 27, substituting it once under vacuum, adding 5ml of omnipaque thereto, and then removing ethanol and moisture under vacuum. This

contrast media for angiography is solidified by absorbing contrast media into chitosan molecular. It also applies to chitosan beads of Examples 17 and 27.

Example 31 : the preparation of chitosan embolic materials solidified to Hydroiodic acid (Hydroiodic acid, Wacopure Chemical, Japan)

Embolic materials, in which hydroiodic acid has been absorbed and solidified, have been obtained by adding 3ml of ethanol to 100mg of chitosan embolic material of Examples 17, 26, and 27, substituting it three times under vacuum, adding 5ml of hydroiodic acid having a concentration of 320mg I/ml thereto, removing ethanol and moisture under vacuum, and then washing them by water until neutralized. This contrast media for angiography is solidified by ion exchange between iodine and amino group of chitosan from a combined amino group of chitosan and hydrogen ion of hydroiodic acid. It also applies to chitosan beads of Examples 17 and 27.

Example 32 : Preparing chitosan embolic materials fixed to doxorubicin · HCl (trade name: Ildong-Adriamycin PFS Injection, main ingredients: doxorubicin · HCl, 10mg doxorubicin·HCl/5ml, Ildong Pharmaceuticals Co., Ltd, Korea) or epirubicin · HCl (trade name: Ildong Pharmorubicin PFS Injection, main ingredients: epirubicin · HCl, 10mg epirubicin · HCl/5ml, Ildong Pharmaceuticals Co., Ltd, Inc., Korea)

Embolic materials, in which doxorubicin · HCl has been absorbed and solidified, have been obtained by adding 3ml of ethanol to 100mg chitosan embolic materials of Examples 17, 26, and 27, substituting it twice under vacuum, adding 5ml of doxorubicin · HCl thereto, and then removing ethanol and moisture under vacuum. This contrast media for angiography is solidified by absorbing doxorubicin

· HCl into molecular chains of chitosan. It applies to chitosan bead of Examples 17 and 27 and doxorubicin · HCl has been solidified by the same method.

As stated above, chitin and/or chitosan embolic materials of the present invention are highly biocompatible blood vessel embolic materials, which may embody a perfect embolization without any adverse reactions and a good stability not to flow into the lungs through capillary vessels and which enable obtaining a radiograph for embolic materials themselves by absorbing contrast media into embolic materials, which may achieve revascularization with the lapse of time after embolization due to being degradable in the blood vessel, and which may efficiently occlude the desired size of blood vessels because they are able to be prepared in a relatively uniform size in the specific ranges. Further, they have no oncogenicity and they are favorable to embolization since they generate more or less inflammation in the blood vessels. If an anticancer agent is solidified to blood vessel embolic materials, blood vessel embolic materials which may be controlled the release of an anticancer agent into a uniform concentration for a long hour are prepared, and therefore it is expected to be effectively used for treatment of tumor.

Chitosan bead embolic materials according to the present invention can represent the embolization effect by occluding renovascular and inducing coagulation necrosis. Furthermore, compared with the conventional PVA particles, they show changes in an equal degree in blood test and biochemical examination, and they can be provided at a low cost.

What is claims is:

1. Embolic materials comprising of chitin and/or chitosan.

2. Embolic materials according to claim 1, wherein said embolic materials are substantially spherical chitin and/or chitosan particles having an average diameter of 50 to 1000㎛.

3. Embolic materials according to claim 1, wherein said embolic materials are substantially spherical chitosan beads having an average diameter of 50 to 1000㎛.

4. Embolic materials according to claim 3, wherein said embolic materials are nondegradable in the human body, which is cross-linked by a crosslink agent.

5. Embolic materials according to claim 4, wherein said cross-linking agent is at least one selected from a group consisting of hexamethylenediisocyanate, glutaraldehyde, epichlorohydrine and mixture thereof.

6. Embolic materials according to any one of claims 1 to 5, wherein said embolic materials are absorbed with a contrast media thereto.

7. Embolic materials according to any one of claims 1 to 5, wherein said embolic materials are a control released form of drug in which an anticancer agent is absorbed thereto.

8. Embolic materials according to any one of claims 1 to 5, wherein said embolic materials have the suspending property of at least for 5 minutes in a physiological salt solution, or the same, including said contrast media.

9. Method for preparing chitosan beads embolic materials comprising the step of:

forming a microdroplet by passing through a capillary with a chitosan solution at a state of pressurizing under an inert gas stream around said capillary; and

precipitating said microdroplet into a coagulating solution to obtain chitosan beads.

10. Embolic materials according to claim 9, wherein said chitosan includes a solvent selected from a group consisting of acetic acid, lactic acid and hydrochloric acid, a deacetylation degree thereof is more than 60%, a viscosity thereof is the range of 5 to 86cps, and the concentration thereof is a range of 1.5 to 5.5%(W/V).

11. Embolic materials according to 9 or 10, wherein said coagulating solution is a mixed solvent of sodium hydroxide or potassium hydroxide : ethanol : water of a range of 1 : 0 ~ 18 : 4 ~ 24.

12. Embolic materials according to claim 9 or 10, wherein said beads are cross-linked with a cross-linking agent or absorbed and solidified by a contrast media or anticancer agent.

FIG. 1A

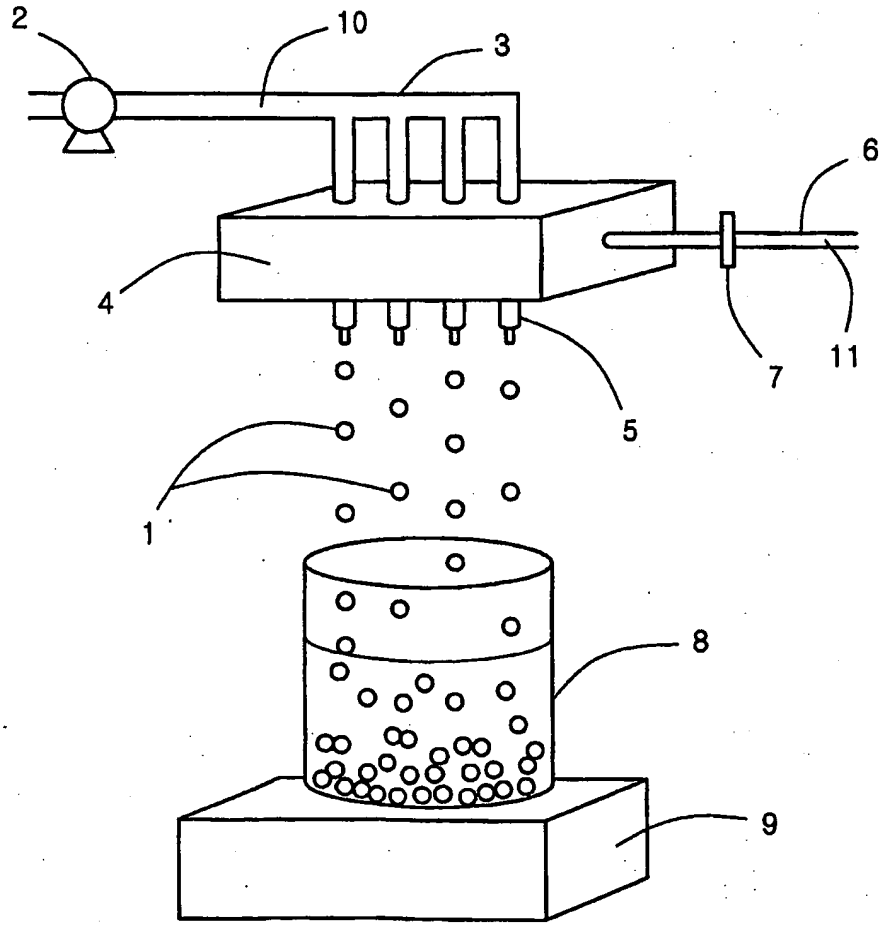


FIG. 1B

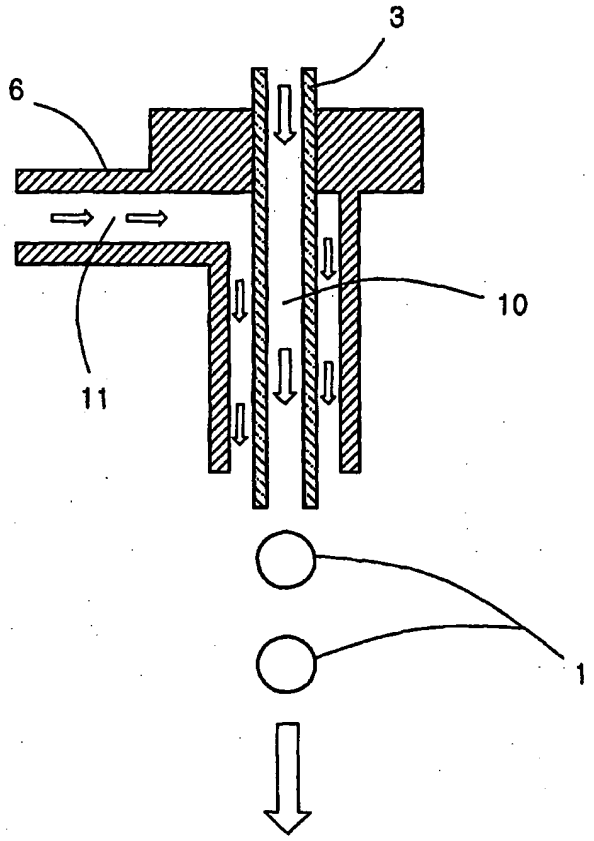


FIG. 2

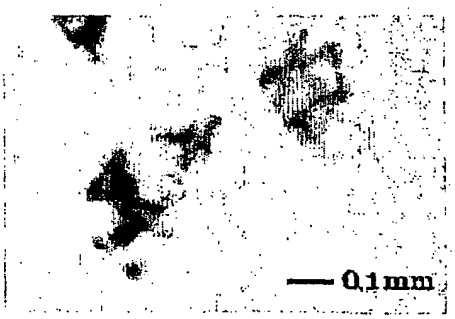


FIG. 3A

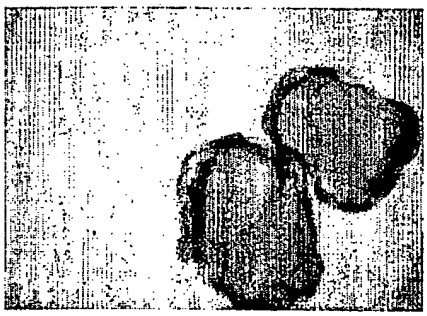


FIG.3B

FIG.4A

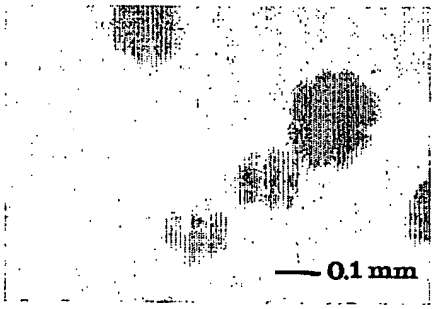


FIG.4B

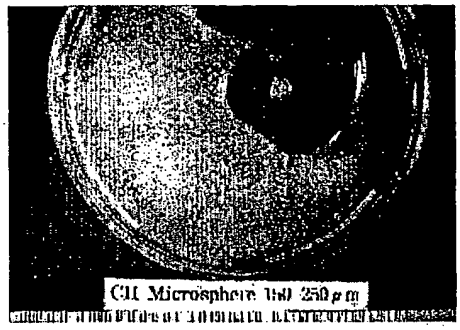


FIG.5

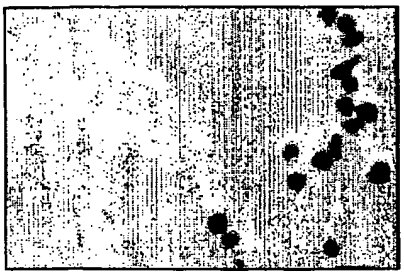


FIG.6A

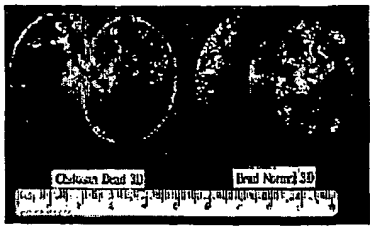


FIG.6B

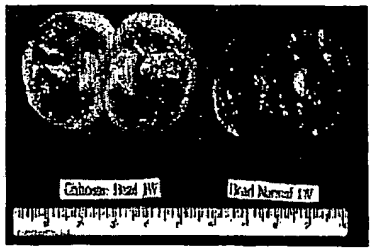


FIG.6C

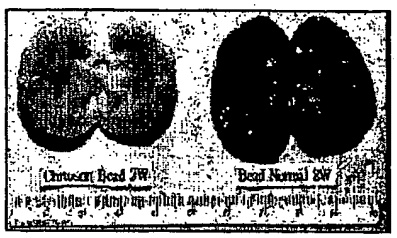


FIG.6D

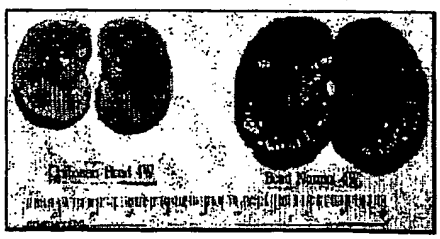
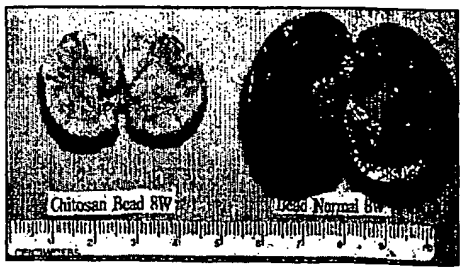


FIG.6E



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR02/01443

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 31/722

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and Applications for inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

MEDLINE, JAPIO, CAPLUS(STN)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	NICHIOKA, YUTAKA; KYOTANI, SHOJIRO 'A study of embolizing materials for chemo-embolization therapy of hepatocellular carcinoma: embolic effect of cisplatin albumin microspheres using chitin and chitosan in dogs, and changes of cisplatin content in blood and tissue' Chemical & Pharmaceutical Bulletin (1992), 40(1), p267-8, SEE ABSTRACT	1
A	WO 2001/30411 A1(KAKEN PHARMACEUTICAL CO., LTD.) 03 MAY 2001, SEE THE WHOLE DOCUMENT	1
A	WO 98/03203 A1(YAMANOUCHI PHARMACEUTICAL CO., LTD.) 29 JANUARY 1998, SEE THE WHOLE DOCUMENT	1

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
28 NOVEMBER 2002 (28.11.2002)	29 NOVEMBER 2002 (29.11.2002)

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR02/01443

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2001/30411 A1	03. 05. 2001	AU 200079578 A5	08. 05. 2001
WO 98/03203 A1	29. 01.1998	AU 9734615 A1	10. 02. 1998